STANDARD OPERATING PROCEDURE		
SOP NO.: GLP-DA-01		Page No.: 1 of 12
Title: **AUDITING FIELD STUDIES - ANALYTICAL CHEMISTRY**		
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1. PURPOSE

To provide guidance and a standard procedure for auditing the analytical chemistry portions (specimen analyses) of pesticide field studies submitted to the Agency in support of applications for research or marketing permits for pesticide products regulated by EPA [Sections 3, 4, 5, 8, 18, and 24(c) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended].

2. SCOPE

This standard operating procedure (SOP) will be used in auditing studies conducted to determine the nature and/or magnitude of residues of pesticides and their metabolites and degradation products in soil, water, crops, the edible tissues of livestock, milk, and eggs. This SOP may also be used when auditing residue chemistry data which are part of other studies, such as hazard evaluation in wildlife, aquatic organisms, and non-market plants and insects; worker reentry protection; environmental fate studies; and pesticide spray drift evaluation.

3. OUTLINE OF PROCEDURES

- Method development and validation
- Stability of test substance, metabolites, and degradation products in sample matrix
- Analytical reference standards for test substance metabolites, and degradation products
- Reference standard solutions
- Test substance characterization, control, and handling
- Sample receipt and storage
- Sample preparation, extraction, and cleanup
- Sample analysis
- Quality control: replicates, controls, reagent blanks

4. REFERENCES

4.1 Pesticide Assessment Guidelines U.S. Environmental Protection Agency, Office of Pesticides and Toxic Substances, Office of Pesticides Programs, Washington, D.C.

Series	Guidelines Title
835	Fate, Transport and Transformation
840	Spray Drift
850	Ecological Effects
860	Residue Chemistry
875	Occupational and Residential Exposure
885	Microbial Pesticides

Standard Evaluation Procedures (SEPs) as appropriate

Standard Operating Procedures (SOPs):

- GLP-C-02, GLP Compliance
- GLP-S-02, Evidence Gathering
- GLP-S-03, Summary Report Format
- GLP-S-04, Full Report Format
- GLP-S-05, Glossary of Terms

5. SPECIFIC PROCEDURE

The audit of any laboratory data in support of field studies should include a review of the following study components, where applicable:

5.1 Method Development and Validation

Part of the audit of an analytical phase of a study should include a review of the analytical methodology used to analyze specimen samples. Method development and validation are not required to be conducted according to the Good Laboratory Practice Standards regulations, except where the results of this work are submitted to the Agency as studies or parts of studies. However, method development and validation are vitally necessary to support any residue study, thus the associated raw data and records must be retained and available for review by the auditor.

FIFRA Books and Records requirement as spelled out in 40 CFR 169.2(k) also dictates retention of these records for the life of a pesticide registration.

The auditor should address the following issues relating to methodology:

- Were standard methods used [i.e., official (AOAC, EPA, FDA) or other recognized analytical methods?] If not, what was the source of the method? Was it appropriate for the intended purpose? Was it sufficiently sensitive, reproducible, and specific for the purpose of the study?

- Was the method, as originally developed, applicable to the types of sample matrices that were analyzed for in the study? Were alterations in the original method made to accommodate different sample matrices, lower detection requirements, or other study conditions?

- Was the method validated by the analytical laboratory for actual analytical conditions? How was the validation conducted? Was the method validated by the same staff that conducted the sample analyses?

- Was the method validated by the laboratory over the entire range of concentrations expected in the study?

- Was the method validated for all expected metabolites and/or degradation products expected in the study and required-by the study protocol?

- Was the method validated for all sample matrices specified by the protocol?

- How were the limit of detection (LOD) and limit of quantification (LOQ) defined and determined? Were these limits appropriate and adequate? Were they properly calculated?

- Were all data generated during method validation retained by the laboratory and available for audit? Were there any

discrepancies or deficiencies? Did the data support the final report?

5.2 Stability of Test Substance, Metabolites, and Degradation, Products in Sample Matrix

Since there is generally a lapse of several days to weeks between the collection of a specimen sample and its analysis, the study personnel must be able to document that the pesticide and, where applicable, its metabolites and/or degradation products were stable in the sample under the conditions of storage for the maximum period that the sample was stored. The auditor must verify that the stability testing was done and that data and records are present and available for audit.

Stability testing may be conducted either prior to the study or concurrently.

The auditor should verify the following:

- Was stability testing performed? What laboratory did the testing? Did the overall study protocol address stability testing or was it a separate study with its own protocol?

- If stability testing was not performed, was there justification for this? Was stability testing conducted using a similar sample matrix rather than the identical sample matrix (i.e., carrots when the study was done on beets, corn fodder when the study was done on sorghum fodder, etc.)? Did this failure to conduct stability testing on the study sample matrix appear to affect the validity of the study test results?

- What fortification levels were used for stability testing? Were they similar to the expected residue levels in study samples?

- Did the time frame of stability testing match or exceed the maximum storage period for the study samples?

- Were fortified samples analyzed using the same methodology employed for the study samples?

- Did the stability testing demonstrate that there was adequate storage stability for the conditions (i.e.,temperature, humidity, etc.) of sample storage and analysis?

- Were all stability data available for audit and consistent with the reported findings?

5.3 Analytical Reference Standards

The analysis and characterization of analytical reference standards (reference substances), as well as the documentation of receipt and distribution, are Good Laboratory Practice (GLP) Standards issues and are covered in detail in SOP No. GLP-C-02, Section 5.5.

It should be noted that analytical reference standards may be obtained from a number of sources including the sponsor, commercial suppliers, custom synthesizers, university and other research laboratories, etc. In some instances, particularly with older materials predating the GLP Standards regulations, the source may not be known. The analysis and characterization of the reference standards may have been conducted by the supplier, who may furnish nothing more than a label statement of percent purity; in addition, the reliability of this analysis may not be known.

Under the new regulations, test facility management is responsible for assuring that the reference standard is appropriately tested for identity and purity, and must either obtain the raw data and records for this testing from the supplier, or must arrange to have the material independently analyzed. In either event, the analyses must be performed under GLP purview, and the raw data and records must be retained and available for audit. During the audit, the audit or will verify the source of the reference standards, review the analytical data supporting the identity and purity of these chemicals, and ascertain if the data were generated according to GLP requirements. When the source of standard cannot be determined, appropriate additional documentation shall be obtained by the inspector, such as personal statements, copies of labels, etc.

Analytical reference standards for pesticide metabolites and degradation products are often difficult to obtain in sufficient

quantity to permit the type of detailed analysis and/or characterization that is possible for the parent pesticide and its more common metabolites. The auditor must exercise professional judgment in addressing the adequacy of any analysis, characterization, and archiving conducted (or not conducted) for these types of standards. The study records should address the source of the metabolite standard(s) and the method(s) of synthesis, if known. They should also contain as much information as possible concerning analysis for identity, purity and stability, and should specifically address any problems or restrictions in obtaining detailed analyses. The inspector and/or auditor shall pay particular attention to reconstruction of the synthesis of radio labeled compounds to assure that the label is in the specified position of the molecule.

In general, if any analyses of analytical reference standards were conducted at the audited facility, the auditor should conduct an audit of the raw data and other records. These analyses are also required to have been conducted under GLP Standards regulations since October 1989, and the underlying raw data and records must be retained.

Additional points to be considered when reviewing the identity and purity of analytical reference standards include:

- Has stability been demonstrated? How long ago were the reported chemical analyses conducted? Were they recent enough to preclude any subsequent significant changes in purity or composition if no stability data were available?

- Were the specific activity and radiochemical purity determined for radio labeled analytical standards? Was stability verified for these compounds, if appropriate?

- Were the reference standards stored so as to minimize degradation? Was labeling adequate to prevent mixup and to meet requirements of 40 CFR sections 160.105 and 160.107.

5.4 Standard Reference Solutions and Instrument Calibration

Several additional issues concerning analytical reference standards will also normally be addressed by the auditor, including

preparation and storage of analytical reference solutions, determination of detector response and detector linearity, and any other factors relating to the reliability and/or validity of the analytical reference standard solutions. When auditing the raw data and records relating to these issues, the auditor should consider the following:

- Were data retained that document the preparation of stock solutions and working dilutions of analytical reference standards? Who prepared the standard solutions? Was there an SOP in place and being followed for preparation and handling of stock and working standard solutions?

- How were standard solutions stored? What kind of containers were being used? Did changes in concentration caused by evaporation of the solvent appear to be minimal? Were standard solutions protected from degradation by light?

- Was proper labeling used to uniquely identify with report to personnel, data, notebook reference, expiration date, etc. to preclude mixup and use of outdated standards?

- How often were fresh working standards prepared. Were practices consistent with applicable SOPs and/or protocols?

- Were standard solutions used exclusively for this study, or were they used for more than one study? Did more than one person appear to have used them? Was there evidence of possible contamination or other loss of integrity for the standard solutions?

- Did the instrument detector response to the analytical standards solution remain relatively stable or did it change with time? If it changed, was there a steady progression to either greater or lesser response, or did the variation appear to be random? Were study personnel aware of any changes in detector response? Was there documentation of the cause and, if appropriate, any remedial action taken?

- Was quantification made from the calibration curve or from single standards? Was there documentation that the detector response was established over the entire range used

for quantification of samples? If the response was not linear, how was this addressed in the calculations?

! How often was a new calibration curve prepared? How often, relative to analysis of samples, was the instrument recalibrated with analytical reference standard(s)?

5.5 Test Substance Characterization, Control, and Handling

Test substance analysis, characterization, stability, solubility, receipt, distribution, and handling are specific GLP Standards issues and are covered in SOP No. GLP-C-02, Section 5.5.

Since radio labeled test substances are often used in chemical fate and metabolism studies, the auditor should be certain that the specific activity and radiochemical purity of the test substance are adequately determined and documented. It is also very important that the synthesis of a radio labeled test substance be fully and adequately documented, since the position of the label in the molecule is usually germane to the study results and adherence to test guidelines.

5.6 Analysis of Test Substance Mixtures

Preparation and analysis of mixtures of test substance with carrier is generally addressed in SOP No. GLP-C-02, Section 5.5.3. Preparation and analysis of agricultural tank mixes is addressed in SOP No. GLP-DA-02.

5.7 Sample Receipt and Storage

The study protocol or facility SOPs should address procedures to be followed for receipt of samples, maintenance of records to track the distribution and disposition of samples, and storage of samples prior to analysis. The auditor must verify that these procedures were adequate and were followed. Review of records and documents, and interviews with study personnel should enable the auditor to answer the following questions:

! What records were maintained to document receipt of the sample(s)? Did the records document date and time of receipt, and condition of the sample(s) upon receipt?

! Was there a logbook or other documentation for the storage location and for the distribution of the sample(s)? Where were the samples stored prior to analysis? If refrigerated or frozen, were there records of temperatures during storage?

! When were samples distributed for analysis? Who obtained them? How much was distributed, and on what occasions? How was surplus material disposed of?

! Was sample preparation (i.e., grinding, sieving, drying, etc.) adequately documented as to when, where, and who performed the operations, particularly if performed by a group separate from the analytical personnel.

! Were storage and handling procedures adequate to ensure the integrity of the sample(s)?

5.8 Sample Preparation, Extraction, and Cleanup

Analytical methodology should specify procedures to be used for sample preparation (homogenizing, mixing, grinding), and isolating the analyte(s) from the sample matrix, usually by extraction and some form of cleanup. The auditor must determine through review of available documentation that this method was followed, and that required procedures were followed in the event that deviations from the written and approved SOP or protocol occurred. Among the areas to be reviewed by the auditor are:

! Who prepared and extracted the sample?
! How were data recorded? Were worksheets and/or notebooks properly used, with dates and identification of technicians recorded?
! Were balances calibrated? How often and by whom? Were calibration procedures appropriate for their use in the analyses?
! When sample preparation and analysis could not be completed in 1 day, how and where were samples and/or extracts stored? Did the raw data adequately document storage conditions?
! How much time elapsed between receipt of samples and subsequent analysis? How much time elapsed between the

beginning of sample preparation and final quantitative or qualitative measurement? If delays occurred, were reasonable explanations offered? Were delays the possible result of too few technicians or analysts? Were delays caused by instrument or facility problems? Were the quality assurance unit and study director aware of unusual delays? Was stability data generated or available that covered the encountered delays?

5.9 Sample Analysis

Quantitative and/or qualitative analysis is normally the final technical phase of a residue study. The auditor must determine that this was conducted, as described in study analytical methodology and facility SOPs. Initially, at least 10% of the analytical data should be audited. When discrepancies, data gaps, or other problems are encountered, additional data (up to 100%) should be audited, in order to determine the magnitude of any data problems. The auditor should also verify that all standards of good analytical practice are followed. At a minimum, the data audit should address the following:

- Were there any changes in procedure from that given in the protocol and/or SOPs. Were these properly documented?
- Were instruments always properly calibrated? How was this documented?
- Was automated data collection (ADC) used? Were hard copies of the data collected in this manner archived with other study data? Were tapes and/or discs also archived? Were there written SOPs for ADC procedures and data storage?
- Were SOPs or protocol directions available to define criteria for: (1) re-analysis of samples; (2) number of significant figures to be reported; (3) rounding; and (4) reporting analytical values that were less than the limit of quantification and less than the limit of detection?
- Were analytical reference standards analyzed concurrently and at subsequent appropriate intervals? Were calculations made using linearity curves, or from single reference points?

- Was it possible to reconstruct the study from the raw data and other records? Was it possible to follow manipulations of data, application of correction factors, averaging of results from replicate analyses, and other procedures used to produce derived or summarized data, tables, or figures.
- Did the quality assurance unit review the analytical results? Was the review adequately documented and reported? Did the study report contain errors that should have been found during internal reviews by study personnel, quality assurance unit or the study director?
- What percent of reported data and results were verified by the EPA auditor? Were all necessary data and records available for audit? Were any discrepancies noted between raw data and reported study results? Were any irregularities noted? Were they few and sporadic, or significant in number and/or importance?
- Were all the analytical data which were generated during the study used in the final report? If some data were not used, what was given as the reason? Was the reason scientifically valid? Could the study conclusions have been affected by the exclusion of these results?

5.10 Quality Control: Replicates. Controls. and Reagent Blanks

Because of the potential for analytical problems in residue determinations, a certain amount of quality control (QC) should be conducted concurrently with the study sample analyses. This QC usually takes the form of some or all of the following: (1) periodic analysis of replicate samples to determine reproducibility of analytical results; (2) analysis of controls (samples from portions of the test system which were not treated with the test substance) to determine potential interference from the sample matrix, containers, sampling equipment, or from other contamination introduced from soil, water, agricultural practices, or other sources during the conduct of the field portion of the study; (3) analysis of reagent or blanks to determine interference or contamination from the reagents or glassware used in the analyses; and (4) analysis of control samples which have been fortified with known quantities of the analyte(s) to determine the analytical efficiency of the methodology.

The study protocol and/or SOPs should define which of the above QC procedures are to be included with the study, and how often the procedures are to be conducted. The auditor should verify that the requirements of the protocol and/or SOPs are met. If no or inadequate QC procedures were conducted in conjunction with the study, the auditor should determine the reasons and make an evaluation of the significance of this lapse. Failure to perform proper quality control could seriously compromise the study results.

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